CHARACTERIZATION OF GROWTH FACTORS IN THE PRIME HD DBM™ ALLOGRAFT

MTF Biologics, Research & Development
125 May Street, Edison, NJ 08837, USA

INTRODUCTION

Prime HD DBM™ allograft, processed by MTF Biologics, is a pre-hydrated, ready-to-use, bone void filler. It is intended for the treatment of musculoskeletal defects in a variety of surgical applications where the availability of autogenous bone is limited or the risk of an additional surgical site for harvesting autogenous tissue precludes autograft as an option. As a biologic alternative, Prime HD™ contains many of the same components as autogenous bone necessary for the bone healing process, including the osteoinductive growth factors and the osteoconductive extracellular matrix.

Prime HD DBM™ is composed of elongated demineralized bone (DBM) particles that function as a scaffold for cell attachment and remodeling. A key characteristic of the DBM is that endogenous osteoinductive growth factors in the bone matrix are exposed through a demineralization process, whereby the inorganic mineral in the cortical bone is removed while the organic collagen matrix is retained. Thus, cells which attach to the scaffold not only have an osteoconductive material on which to expand and remodel, but are also able to more effectively interface with local growth factors on the surface, resulting in a positive synergistic effect on bone formation.

These intrinsic properties of Prime HD DBM™ act in concert to support the overall bone healing process and are only maintained in demineralized bone that has been minimally processed in a way that preserves the biological integrity of the tissue. Traditional bone processing and sterilization techniques are harsh and result in irreparable damage to growth factors; this subsequently diminishes osteoinductivity in the demineralized bone[1,2]. MTF Biologics is able to avoid this issue through its proprietary, validated processing method which limits exposure of the bone to harsh processing chemicals, thereby retaining native growth factor activity in Prime HD DBM™. Furthermore, MTF Biologics follows stringent procedures for aseptic processing, eliminating the need for growth factor-damaging terminal sterilization.

The study detailed below presents evidence of growth factor presence in Prime HD DBM™, obtained from immunohistochemical (IHC) staining of the elongated DBM particles. As a whole, these results suggest that MTF Biologics' processing methods successfully preserve the osteoinductive elements of the allograft, representing a potential clinical advantage in the use of Prime HD DBM™ over competitive bone grafting materials.

HISTOLOGICAL EVALUATION OF ENDOGENOUS GROWTH FACTORS

In this study, Prime HD DBM™ was aseptically processed from the cortical bone of three distinct donors using proprietary methods. A cleaning procedure removed residual lipids, blood, and other potentially immunogenic factors from the bone. The cortical bone was then milled and minerals were removed from the tissue via an acid extraction procedure. Rinsing and buffering steps followed to ensure neutral pH of the demineralized bone particles. At the time of sampling, the tissue was placed in a fixative solution, and shipped to an external lab to be embedded, sectioned, and stained for a panel of growth factors known to be relevant to bone healing (BMP-2, BMP-7, PDGF-BB, FGF-1, FGF-2, IGF-1, TGF-β, VEGF).

Results of this IHC staining are visualized in Figure 1 using conventional microscopy imaging. In these images, growth factor presence in Prime HD DBM™ is represented by brown coloration, which can be seen throughout the DBM particles. A negative control, which was not exposed to primary antibody during the staining process, was also prepared from DBM samples for comparison.

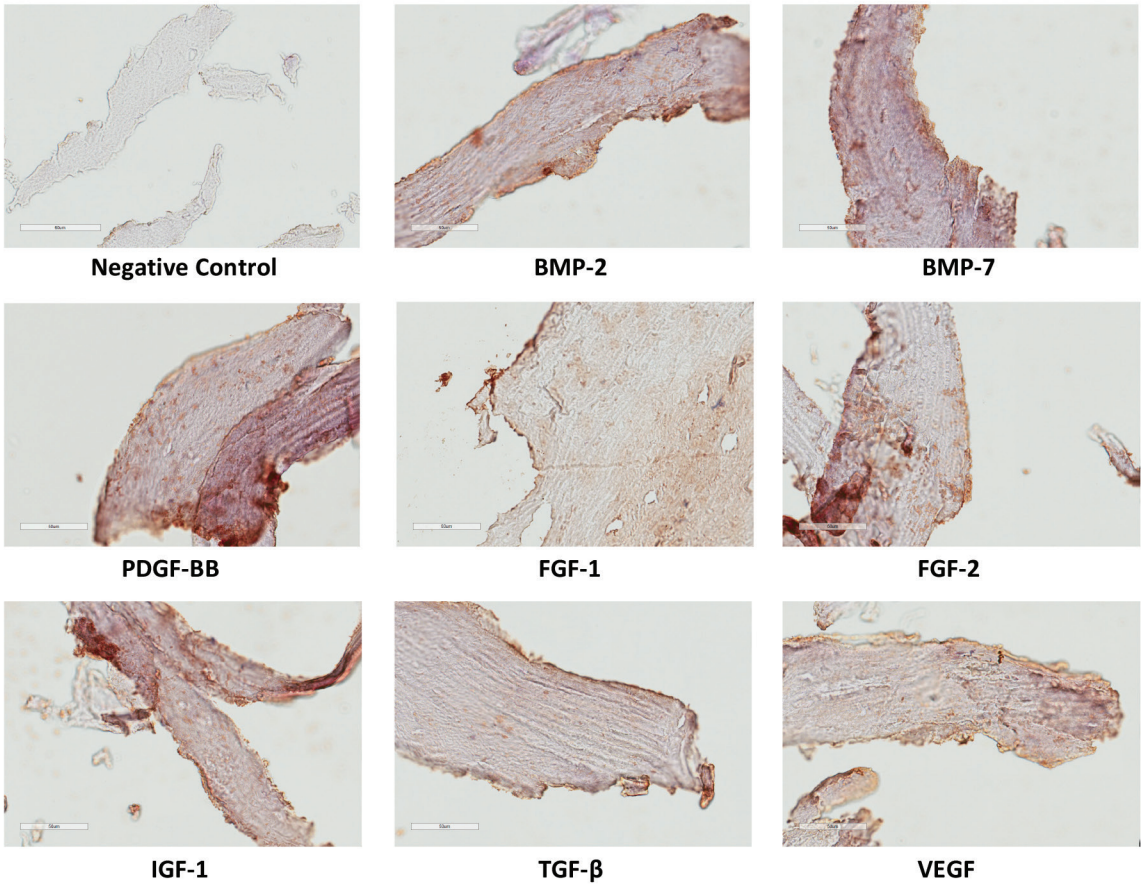

Figure 1: Immunohistochemical staining (DAB-Brown) of growth factors present in Prime HD™

All growth factors expressed positive staining, indicating they were present in the Prime HD DBM™ particles after processing. Table 1 summarizes these results and presents brief descriptions of the role played by each growth factor in bone healing. All growth factors assessed in this study are known to contribute to the bone repair process and the expression of these growth factors varies during the different phases of the bone healing cascade.

Table 1: Growth factors found in MTF Prime HD DBM™ and their respective roles in the bone healing cascade[3,4]

Growth Factor	Role In Bone Healing Cascade	Presence
BMP-2	Differentiation of MSCs into osteoprogenitor cells, chondrocytes and osteoblasts	✓
BMP-7	Differentiation of osteoprogenitor cells into osteoblasts	✓
PDGF- BB	Mitogenic for MSCs and osteoblasts and responsible for macrophage chemotaxis	✓
FGF-1	Mitogenic for MSCs, chondrocytes, and osteoblasts. Promotes vascularization.	✓
FGF-2	Mitogenic for MSCs, chondrocytes, and osteoblasts. Promotes vascularization.	✓
IGF-1	Promotes proliferation and differentiation of osteoprogenitor cells	✓
TGF-β	Pleiotropic growth factor responsible for stimulation of undifferentiated MSCs	✓
VEGF	Promotes migration and proliferation of osteoblasts. Promotes angiogenesis.	✓

RECOMBINANT GROWTH FACTOR BIOLOGICS & DEMINERALIZED BONE

Within the broad range of options for bone grafting materials currently available, two major categories of products containing osteoinductive growth factors exist: (1) biologics dosed with recombinant growth factors and (2) demineralized allograft bone grafts such as Prime HD DBM™ which have been minimally processed to expose endogenous factors.

Technologies incorporating recombinant growth factors include InFuse® (rhBMP-2) and Augment® (rhPDGF-BB), with InFuse® having seen the most widespread usage for spinal fusion procedures. Although InFuse® has been shown to aid in the bone healing process[5], there have also been noted problems associated with its use, including ectopic bone growth and evidence of increased cancer risk[6]. While the exact causes of these issues are difficult to determine, the use of exogenous growth factors at concentrations hundreds of times higher than physiological levels may play a role in eliciting undesired and potentially harmful responses from host cells and tissue.

In contrast to such recombinant biologic products that provide a supraphysiologic dosage of a single growth factor, Prime HD DBM™ contains a broad spectrum of endogenous growth factors identified as necessary in the bone healing cascade (BMP-2, BMP-7, PDGF-BB, TGF-β, IGF-1, FGF-1, FGF-2, and VEGF) and is minimally processed to expose these osteoinductive factors at a normal, physiological level. Moreover, while the recombinant growth factors in biologic products are released in a single large dose, the endogenous growth factors in demineralized bone are slowly released over time as the demineralized matrix is remodeled[7].

THE MTF BIOLOGICS ADVANTAGE

Amongst demineralized bone grafts, many types are commercially available from various tissue banks and the demineralized matrix of each can differ considerably with regard to their osteoinductive potential. Several factors may account for such variability, including the quality of the starting tissue and the processing techniques utilized. These vary widely from bank to bank because certain standards for industry donor selection criteria are voluntary, while regulations governing processing practices can be met through a variety of means, some of which severely compromise the osteoinductive components of bone tissue.

Rather than risk providing grafts of less-than-optimal quality that may lead to less-than-optimal clinical outcomes, MTF Biologics aims to provide the highest quality tissue by following the most stringent standards in the industry. MTF Biologics' quality and safety criteria consistently meet or exceed the best practice standards of the American Association of Tissue Banks (AATB), as well as the guidelines for screening and testing of tissue donors set forth by the Food and Drug Administration (FDA). With regards to processing, current techniques used by some tissue banks to clean, process, and sterilize demineralized bone have been shown to be detrimental to the quality of the tissue. In contrast, MTF Biologics' proprietary processing techniques are specifically designed to expose the endogenous growth factors in Prime HD DBM™ while preserving the osteoinductive potential of the tissue.

CONCLUSION

Current understanding of bone healing indicates a critical role for key growth factors in effecting osteogenic cell activity and regulating the overall process of new bone formation[3,8,9]. While some biologics have attempted to artificially augment these processes by introducing high concentrations of recombinant growth factors, there is evidence to suggest that use of such products is associated with increased risks of complications and adverse events. Meanwhile, bone allografts, which achieve bioactivity via demineralization, can be of widely varied quality depending on which practices a particular tissue bank chooses to follow.

Prime HD DBM™ remains distinct within both categories of bone-healing products by virtue of the stringent, aseptic techniques with which it is processed and MTF Biologics' commitment to consistently screen for the highest quality donors. The results of these efforts are empirically demonstrated in this study, which establishes that Prime HD DBM™ contains a broad spectrum of endogenous growth factors known to be essential during the bone healing cascade. The presence of these growth factors suggests that the osteoinductivity of the allograft is preserved during processing – a factor which may contribute to successful clinical outcomes for a variety of orthopedic applications.

APPENDIX: BONE HEALING CASCADE

Three components are necessary for bone healing and/or bone graft incorporation: the presence of bone-forming cells, a signal to trigger differentiation of the host cells to bone-forming cells, and a scaffold or matrix on which the new bone can form. When bone fracture or injury occurs, there is a loss of mechanical integrity of the bone and a disruption of the blood supply. The healing cascade begins immediately and occurs in three distinct but overlapping phases: inflammation, repair, and remodeling[8].

Inflammation is the process by which host cells remove debris from the injured site, prepare the local matrix to support cell growth, and enable new bone to be formed; during this phase, revascularization required for new bone growth also begins. Repair includes the recruitment and differentiation of mesenchymal stem cells (MSCs) into osteoblasts which produce new bone at the injured site. Lastly, remodeling is the resorption of immature or extraneous bone coupled with reorientation of bone along the direction of mechanical loading to provide adequate structure support. These phases are regulated by the local release of growth factors which include BMP-2, BMP-7, PDGF-BB, TGF-β, IGF-1, FGF-1, FGF-2, and VEGF. An overview of growth factor involvement in the bone healing cascade is shown in Figure 2.

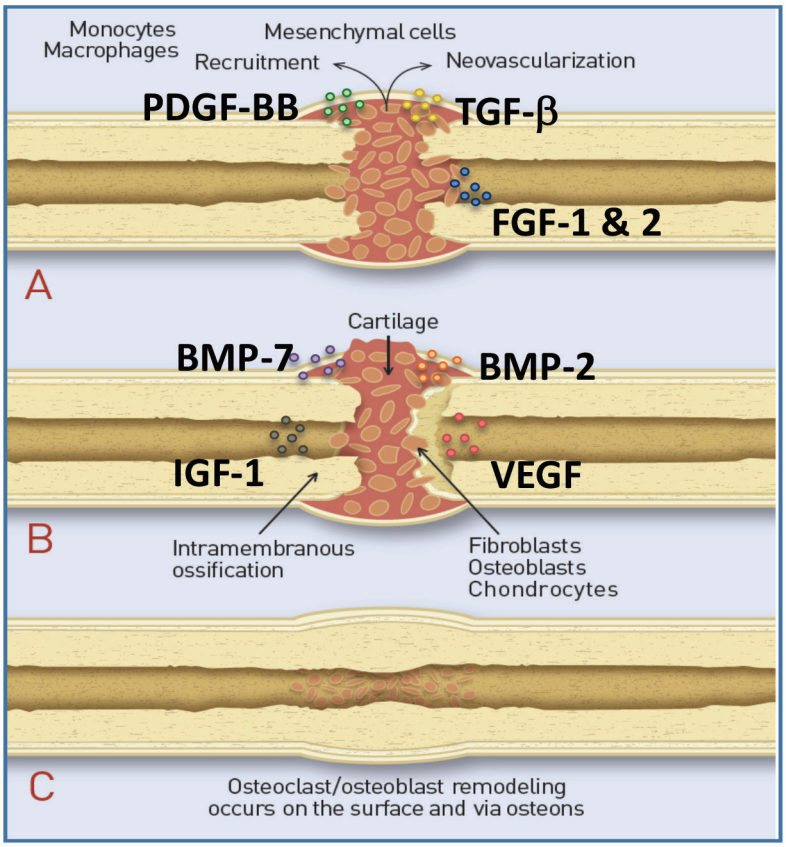

Figure 2: Growth factors involved in the Inflammation (A), Repair (B), & Remodeling (C) phases of the bone healing cascade*

*This graphic is a simplified illustration which shows cytokines only where they are most highly expressed during the bone healing process. The majority of the cytokines shown are continually expressed at varying levels throughout the three major phases of bone healing.

In addition to regulating the overall process of bone healing, osteoinductive growth factors are also essential for the differentiation of MSCs into mature bone-forming osteoblasts. A simplified diagram depicting the expression profiles of various growth factors during osteoblastic differentiation is shown in Figure 3.

Figure 3.

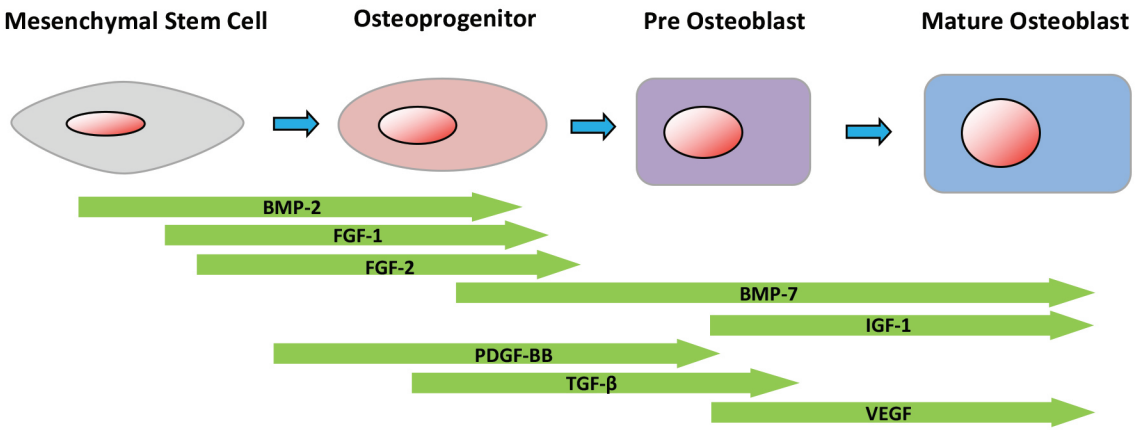

Figure 3: Cascade of various growth factors in the differentiation of osteoblasts for bone repair and remodeling[3,9]

*Adapted from "Effects of growth factors and cytokines on osteoblast differentiation" by Hughes F J, et al. *Periodontology 2000*. 41:48-71.

REFERENCES

1. Gertzman et al. (2005) The effect of cold gamma radiation sterilization on the properties of demineralized bone matrix. In Kennedy JF, Philips GO, Williams PA, editors. Sterilization of tissues using ionizing radiations: CRC Press. Pp 151-161.

2. Han et al. Effects of gamma irradiation on osteoinduction associated with demineralized bone matrix. *JOR*. 2008; 26(1): 75-82

3. Yang Y Q, et al. The role of vascular endothelial growth factors in ossification. *International Journal of Oral Science*. 2012; 4: 64-68

4. Phillips A M. Overview of the fracture healing cascade. *Injury, Int. J. Care Injured.*2005; 36S:s5-S7

5. Carano et al. Angiogenesis and bone repair. *DDT*. 2003; 8(21): 980-989

6. Epstein N E. Complications due to the use of BMP/INFUSE in spine surgery: The evidence continues to mount. *Surg Neurol Int*. 2013; 4(5): S343-S352.

7. Holt et al. Demineralized bone matrix as a vehicle for delivering endogenous and exogenous therapeutics in bone repair. *Advanced Drug Delivery Reviews*. 2012; 64: 1123-1128

8. Kalfas I H. Principles of bone healing. *Neurosurg Focus*. 2001; 10(4): 7-10

9. Hughes et al. Effects of growth factors and cytokines on osteoblast differentiation. *Periodontology 2000*. 2006; 41:48-71